Atty. Dkt. No. 023032/0108

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Joseph ROBERTS *et al.*
Title:	GENETICALLY ENGINEERED GLUTAMINASE AND ITS USE IN ANTIVIRAL AND ANTICANCER THERAPY
Appl. No.:	Unassigned
Filing Date:	April 27, 2001
Examiner:	Unassigned
Art Unit:	Unassigned

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified Application, Applicant respectfully requests that the application be amended as follows:

IN THE SPECIFICATION:

On page 1, insert new title and paragraph after title.

(New) **Cross Reference to Related Application**

(New) This application is a divisional of pending U.S. Application Serial No. 08/050,482, filed April 25, 1995, which is a national stage of PCT/US92/10421 filed December 4, 1992.

Page 7, delete the 4th paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.121.

Figures 1A-1D show the nucleotide and deduced amino acid sequence of the *Pseudomonas 7A* glutaminase gene (SEQ ID NOS 1 & 2. The top strand of the coding DNA sequence is shown from 5′ - 3.′ The numbers shown indicate nucleotide base pairs. The deduced peptide sequence is shown below the DNA sequence. The engineered N-terminal methionine residue is not shown.

Page 8, delete the 1st paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.121.

Figures 2A and 2B depict the sequencing strategy for the *Pseudomonas 7A* glutaminase gene. Figure 2A: Map of the *P7A* glutaminase showing selected restriction sites, the shaded area depicts the region encoding the actual gene product. Hatch marks represent 100 bp. Arrows below this figure show the approximate positions and orientations of sequencing primers with their accompanying names. The arrows with stops indicate the extent and direction of individual sequencing experiments. Figure 2B: Names, sequences, and coordinates of sequencing primers are shown (SEQ IS NOS 3-11). Numbering is from the AAG encoding the N-terminal lysine residue.

Page 23, delete table 1, and replace this table with the following in accordance with 37 CFR §1.121.

Table 1. OLIGONUCLEOTIDE PROBES USED FOR DETECTING THE GLUTAMINASE GENE

Peptide Sequence (1-5) of Sequence SEQ ID NO: 2	NH_2-Lys-Glu-Val-Glu-Asn
Probe A (SEQ ID NO: 2) (14-mer x 32)	AA (AG) GA (AG) GT (TCAG) GA (AG) AA
Peptide Sequence (161-166) of SEQ ID NO: 2	Met-Asn-Asp-Glu-Ile-Gln
Probe B (SEQ ID NO: 13) (18-mer x 48)	ATGGA (TC) GA (TC) GA (AG) AT (TCA) GA (AG)
Peptide Sequence (332-36) of SEQ ID NO: 2	Ile-Phe-Trp-Glu-Tyr-COOH
Probe C (SEQ ID NO: 14) (14-mer x 12)	AT (TCA) TT (TC) TGGGA (AG) TA

Page 26, delete table 3, and replace this table with the following in accordance with 37 CFR §1.121.

Table 3: Oligonucleotides Used in Construction

of a High Level Expression Plasmid

Primers for thermocycle amplification mutagenesis of the glutaminase:
N-terminus (SEQ ID NO: 15)
GCCGGATACCA TATGAAGGAA GTGGAGAACC AGCAG

Internal SalI site (SEQ ID NO: 16)
GCGCGGATCC GTCGACGCCA ACCTTGGCAG

Mutagenized N-terminus of the glutaminase (SEQ ID NOS: 17 & 18)
GGATCCAT ATG AAG GAA GTG GAG AAC
Met Lys Glu Val Glu Asn. . .

Oligonucleotides for *tac* promoter
top (SEQ ID NO: 19):
AGCTTACTCC CCATCCCCCT GTTGACAATT AATCATCGGC TC
GTATAATGTG TGGAATTGTG AGCGGATAAC ATTTCACAC AGGAAACAG
Bottom (SEQ ID NO: 20):
GATCCTGTTT CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACA
TTATACGAGC CGATGATTAA TTGTCAACAG GGGGATGGG AGTA

Filled in product of pME18 (SEQ ID NOS: 21 & 22)

	lacO			S.D.		S.D.		
5'	AATTGTGAGCGGATAACAATTTCACAC			AGGA	AAC	AGGATCCATAT	ATG	AAG
							Met	Lys
GAA	GTA	GAG	AAC 3'					
GLU	Val	Glu	Asn					

Page 31, at the end of the specification, before the claims, delete the Sequence Lising previously filed and insert the printed Sequence Listing submitted in parent application Serial No. 08/050,482 filed April 25, 1995. Please renumber pages 37, 38 and 44 of the specification as pages 42, 43 and 49, respectively.

IN THE CLAIMS:

Please cancel claims 1-23 and 31-46 without prejudice and disclaimer and amend the claims as follows:

In accordance with 37 CFR §1.121, please substitute for original claims 27 and 28 the following rewritten versions of the same claims, as amended. The changes are shown explicitly I the attached "Version with Markings to Show Changes Made."

27. (Amended) The preparation of claim 24, which is free of non-glutaminase *Pseudomonas* proteins.

28. (Amended) The preparation of Claim 24, which is made by the process of:

culturing a recombinant microorganism which comprises a nucleotide sequence that codes for a therapeutically suitable glutaminase; and

collecting said therapeutically suitable glutaminase produced by said microorganism.

47. (New) The preparation of claim 29, wherein said glutaminase is encoded by the nucleotide sequence of SEQ ID NO:1.

48. (New) The preparation of claim 24 wherein said glutaminase has a K_m of

10^{-6} to 10^{-4} M for its reactants and remains active in human sera.

49. (New) The preparation of claim 24, wherein said microorganism is a bacterium.

50. (New) The preparation of claim 49, wherein said microorganism is *E. coli*.

51. (New) A method of producing a therapeutically suitable glutaminase comprising:

culturing a recombinant microorganism which comprises a nucleotide sequence that codes for a therapeutically suitable glutaminase; and

collecting said therapeutically suitable glutaminase produced by said microorganism.

52. (New) A method according to claim 51, wherein said microorganism is a bacterium.

53. (New) A method according to claim 52, wherein said microorganism is *E. coli*.

54. (New) A method according to claim 51, wherein said nucleotide sequence encodes the protein of SEQ ID NO: 2.

55. (New) A method according to claim 54, wherein said protein is encoded by the nucleotide sequence of SEQ ID NO: 1.

56. (New) A method according to claim 51 wherein said glutaminase has a K_m of 10^{-6} to 10^{-4} M for its reactants and remains active in human sera.

57. (New) A method according to claim 51, wherein said glutaminase is a *Pseudomonas* glutaminase.

REMARKS

Claims 1-23 and 31-46 have been cancelled, without prejudice or disclaimer. Claims 24-30 and 47-57 are presented for examination on the merits. Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

Should there be any questions or comments regarding this application, the Examiner is invited to contact the undersigned at the telephone number shown below.

The Examiner is hereby authorized to charge any deficiency or credit any overpayment to our Deposit Account No. 19-0741.

Respectfully submitted,

By _____

Date April 26, 2001

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Attorney for Applicants
Reg. No. 29/768

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Marked up replacement paragraph:

[Figure 1 shows] Figures 1A-1D show the nucleotide and deduced amino acid sequence of the *Pseudomonas 7A* glutaminase gene (SEQ ID NOS 1 & 2. The top strand of the coding DNA sequence is shown from 5' - 3.' The numbers shown indicate nucleotide base pairs. The deduced peptide sequence is shown below the DNA sequence. The engineered N-terminal methionine residue is not shown.

[Figure 2 depicts] Figures 2A and 2B depict the sequencing strategy for the *Pseudomonas 7A* glutaminase gene. Figure 2A: Map of the *P7A* glutaminase showing selected restriction sites, the shaded area depicts the region encoding the actual gene product. Hatch marks represent 100 bp. Arrows below this figure show the approximate positions and orientations of sequencing primers with their accompanying names. The arrows with stops indicate the extent and direction of individual sequencing experiments. Figure 2B: Names, sequences, and coordinates of sequencing primers are shown (SEQ IS NOS 3-11). Numbering is from the AAG encoding the N-terminal lysine residue.

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Marked up rewritten claims:

27. The preparation of claim **[23]** **24,** which is free of **[other]** **non-glutaminase** *Pseudomonas* proteins.

28. The preparation of claim **[23]** **24,** which is made by the process of:

culturing a recombinant microorganism **[comprising a DNA sequence encoding]** **which comprises a nucleotide sequence that codes for** a therapeutically suitable glutaminase; and

collecting **[proteins]** **said therapeutically suitable glutaminase** produced by said microorganism.